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TITLE: Turning Chemopreventive Agents Against Breast Cancer: Sensitizing Cancers to Therapeutics While Protecting Normal Tissues from Toxicity

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14. ABSTRACT
The chemopreventive agent, sulforaphane (SFN) induces, Nfr-2-stabilization and Nrf2-dependent transcription in breast cancer cells. These effects of SFN are dependent upon PKC and PI3K/AKT pathways as well as the likely involvement of direct interactions between SFN and Keapl. SFN sensitizes breast cancer cells to the cytotoxicities of doxorubicin and peclitaxel. Co-treatment with the PKC inhibitor, Ro-31-8221, but not the PI3K inhibitor, LY294002, augments this sensitization by SFN. However, the utility of combined chemopreventive agent (SFN). And cancer drug (doxorubicin and paclitaxel treatment in breast cancer is potentially limited because SFN also sensitizes some non-cancerous cells to cancer drug toxicity.

15. SUBJECT TERMS- chemoprevention, Chemosnsitization, breast cancer therapeutics

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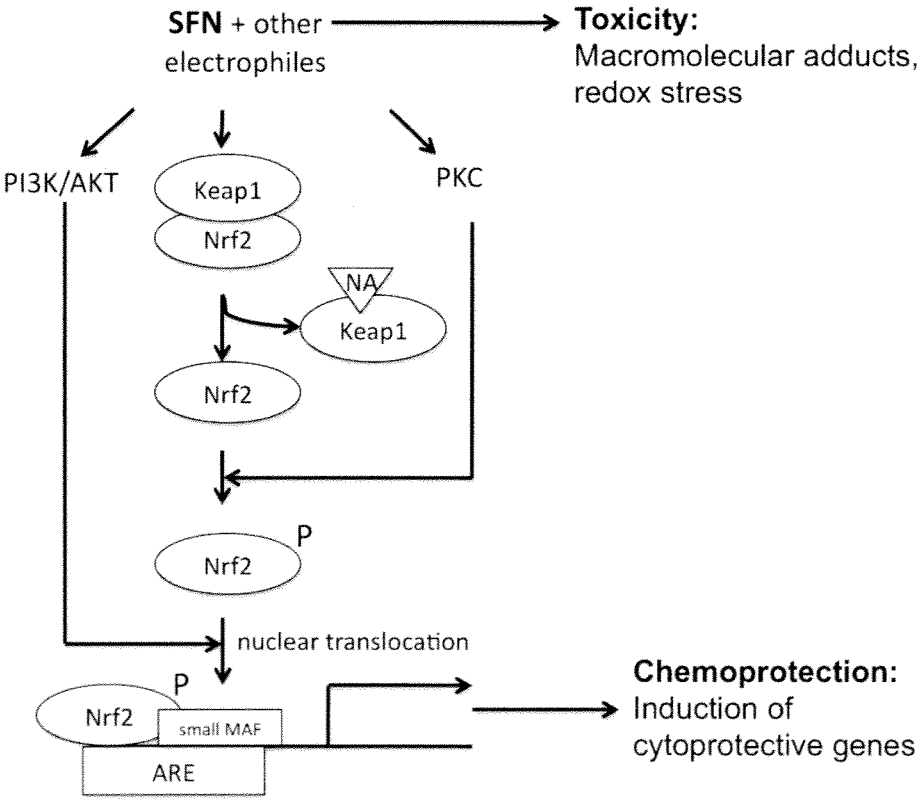
INTRODUCTION

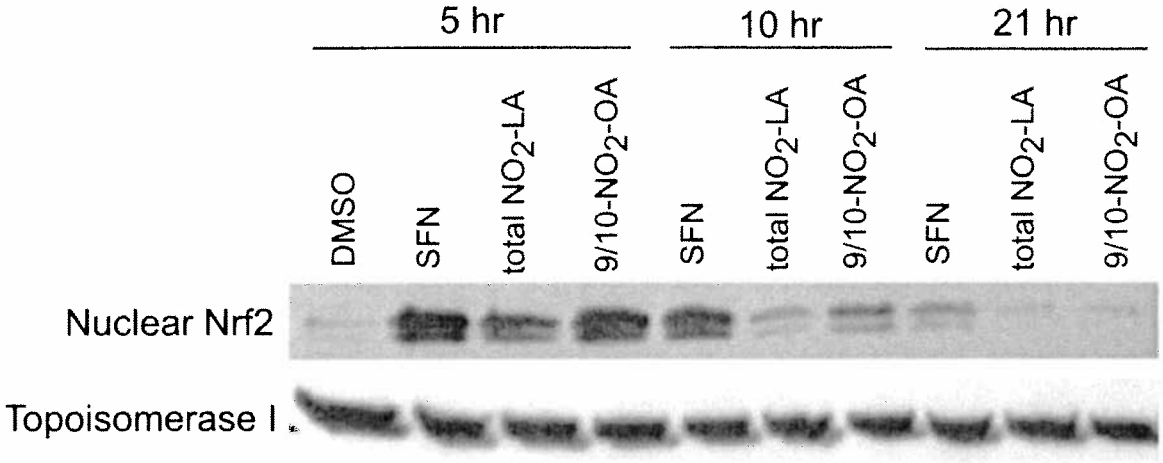
Dietary or nutriceutical chemopreventive agents, such as sulforaphane (SFN), activate the coordinated induction of a large set of gene products that protect the cell from a variety of external toxins including carcinogens and chemotherapeutic drugs. While chemopreventive agents have the potential to protect normal tissues from carcinogens, they can also activate similar protective pathways in established cancers rendering these tumors resistant to therapeutic drugs. Many of these chemopreventive agents act via a variety of converging signaling mechanisms to activate coordinated Nrf2-dependent transcription of a large cassette of genes which, in turn, serve to protect the cell from a variety of chemical toxins and insults Here, it is hypothesized that, because many signaling pathways are dysregulated and otherwise aberrant in breast cancer, not all of the converging pathways leading to Nrf2-dependent transcription activation in normal cells will be operative in breast cancer cells. Thus it is proposed that judicious use of signaling pathway inhibitors can—by inhibiting the limited pathways available to these cancer cells for Nrf2 activation—preferentially disrupt the chemopreventive response in breast cancers while leaving intact the beneficial, redundant Nrf2-activating pathways in normal tissues. Using representative human breast cancer cell lines and non-cancerous cells derived from human breast epithelium, we examined the relative sensitivity of the cells to the chemotherapeutic agents, doxorubicin and paclitaxel, following pre-treatment or continuous co-treatment with SFN.



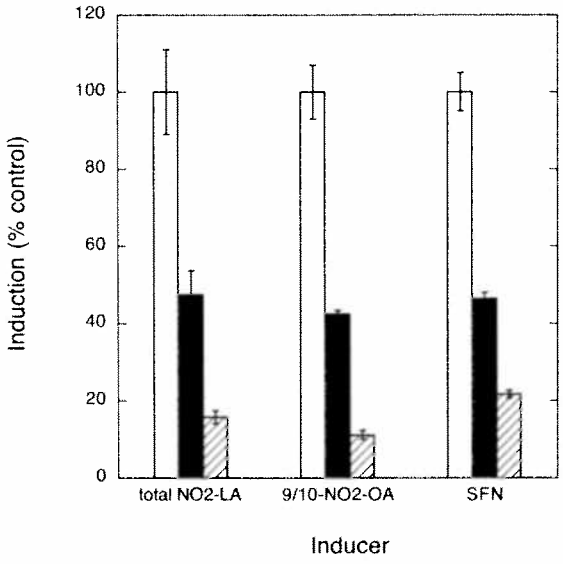
Alternative outcomes of SFN exposure: Toxicity vs Cytoprotection

BODY

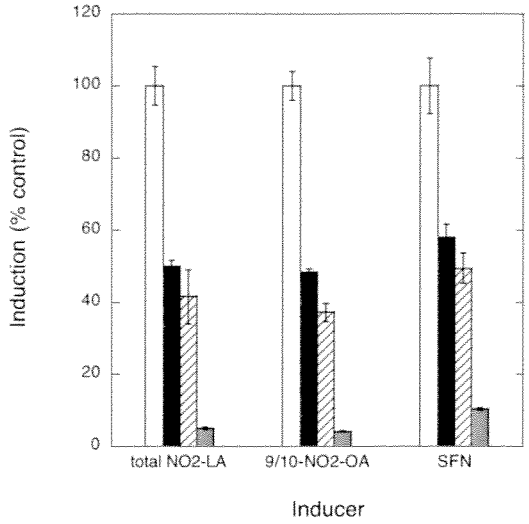
Pilot experiments showed that the chemopreventive electrophile, SFN, as well as the endogenous electrophiles, nitroalkene fatty acids (9/10-nitrooleic acid [9/10-NO_2-OA] and total & 12-nitrolinoleic acids [12-NO_2-LA]) activated Nrf2/ARE-dependent transcription in MCF7 breast cancer cells. Transcription induction was associated with stabilization and accumulation of nuclear Nrf2.



This robust induction of Nrf2/ARE dependent transcription was dependent upon PI3K/AKT and PKC pathways as evidenced by attenuation of induction by the PI3K inhibitors, 0.5 μM wortmannin (black bars) or 25 μM LY294002 (cross-hatched bars) [n.b: no inhibitor (open bars)],



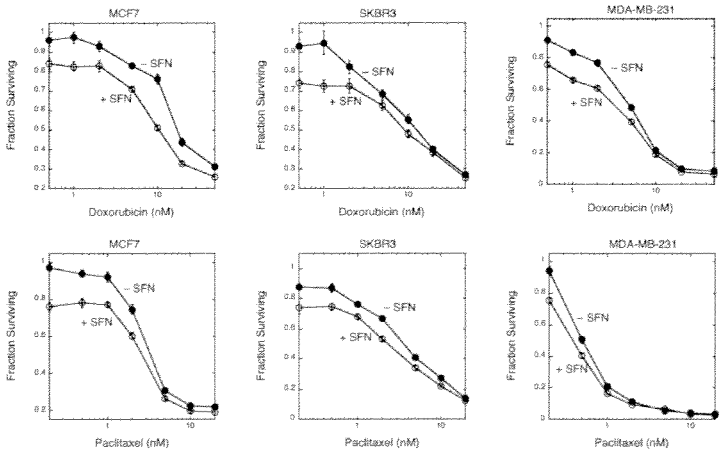
and by attenuation of induction by the PKC inhibitors, staurosporine (15 nM, black bars; 50 nM cross-hatched bars), Ro-31-8220 (shaded bars). No inhibitor controls are shown as open bars:



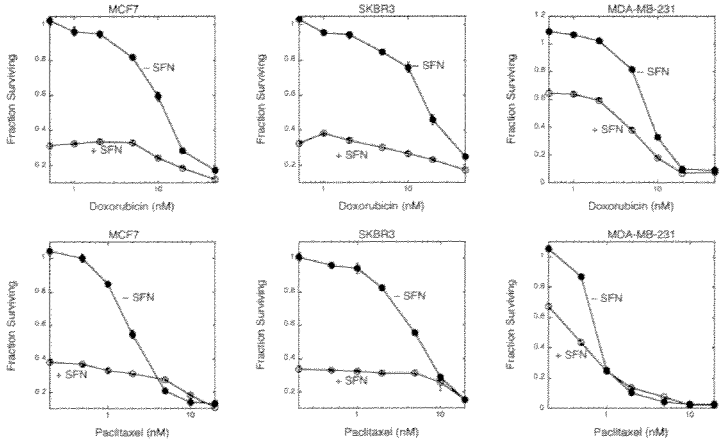
Using representative human breast cancer cell lines and non-cancerous cells derived from human breast epithelium, we examined the relative sensitivity of the cells to the chemotherapeutic agents, doxorubicin and paclitaxel, following pre-treatment or continuous co-treatment with SFN. Breast cancer cells (MCF7, SKBR3 and MDA-MB-231) are *sensitized* to the cytotoxicities of chemotherapeutic drugs by prior and co-treatment with SFN.

SFN sensitizes breast cancer cells to doxorubicin and paclitaxel cytotoxicity

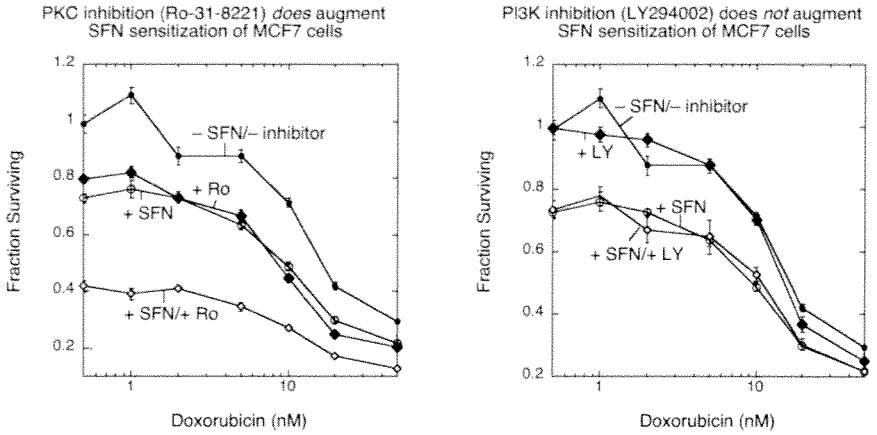
10 uM SFN pulse pre-treatment:



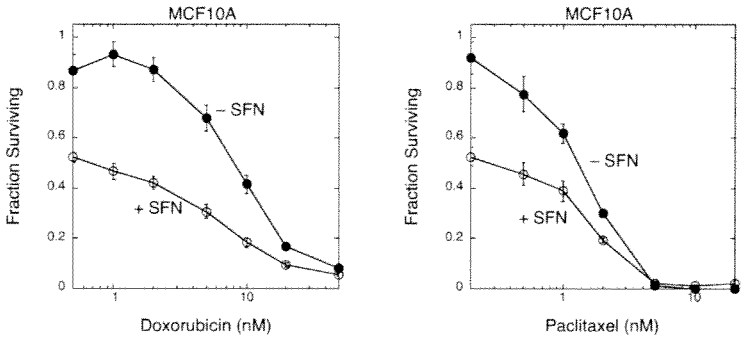
5 uM SFN continuous co-treatment:



Moreover, co-treatment of MCF7 cells with SFN and inhibitors of PKC (Ro-31-8220) but not PI3K (LY294002)—pathways implicated in SFN-mediated induction of the Nrf2-dependent chemopreventive response—further sensitized these cells to the cytotoxicities of chemotherapeutic drugs beyond that achieved with either SFN or inhibitor alone.



However, pre-treatment of a non-cancer cell line derived from human breast epithelium, MCF10A, with SFN also resulted in sensitization to doxorubicin and paclitaxel, suggesting that SFN may augment toxicity of chemotherapeutic drugs.



This lack of specificity of chemosensitization towards cancer cells raises concerns about the potential utility of combining chemopreventive agents like SFN with chemotherapeutic drugs in the treatment of breast cancer.

KEY RESEARCH ACCOMPLISHMENTS

- We demonstrated that the chemopreventive, sulforaphane (SFN), mediates robust stabilization of Nfr2 and its nuclear accumulation in breast cancer cells.

- We demonstrated that Nrf2 accumulation is associated with robust, AKT/PI3K- and PKC-dependent transcription activation of ARE-containing reporter and endogenous cytoprotective genes.

- Pre-treatment or co-treatment of breast cancer cells with SFN sensitizes these cells to the cytotoxicities of chemotherapeutic drugs, doxorubicin and paclitaxel.

- Addition of the PKC inhibitor, R0-31-8221, but not the PI3K inhibitor, LY294002, augment this sensitization by SFN in MCF7 breast cancer cells.

- However, SFN also sensitizes a non-cancer breast-derived cell line, MCF10A, to doxorubicin and paclitaxel toxicity raising concerns that the lack of cancer-targeting specificity of SFN chemosensitization may limit the utility of this approach in the treatment of breast cancer.

REPORTABLE OUTCOMES

1. Darcy J. P. Bates, Pamela K. Smitherman, Alan J. Townsend, S. Bruce King, and Charles S. Morrow. Nitroalkene fatty acids mediate activation of Nrf2/ARE-dependent and PPARγ-dependent transcription by distinct signaling pathways and with significantly different potencies. *Biochemistry* 50: 7765-7773, 2011.

2. Charles S. Morrow. Effect of the chemopreventive agent, sulforaphane (SFN) on relative responses of breast cancer versus normal cells to chemotherapeutic agents. Abstract/Poster to be presented at the Era of Hope Breast Cancer Meeting in Orlando, August, 2011.

CONCLUSION

These data reported herein suggest that 1) SFN may enhance the anti-breast cancer effect of chemotherapeutic drugs and 2) inclusion of PKC targeting inhibitors may augment this effect—the latter, perhaps, via inhibition of SFN-mediated induction of chemoprotective genes while preserving the toxicity of SFN towards cancer cells. However, SFN treatment also results in robust sensitization to chemotherapeutic drugs of an immortalized, non-cancerous breast-derived cell line, MCF10A—a finding that raises serious concerns about the lack of cancer-targeting selectivity and, hence, utility of the combined use of a chemopreventive agent and an anti-cancer drug as an approach to breast cancer treatment.